

U.S./CANADA SARCOMA INTERGROUP ONCOLOGY GROUP

**A PHASE II SURGICAL TRIAL OF INTRALESIONAL RESECTION OF STAGE IA
CHONDROSARCOMA OF BONE**

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1.0 OBJECTIVES

- 1.1 To assess local complications associated with intralesional resection (fracture, nerve palsy, deep venous thrombosis, unexpected rehospitalization, unanticipated re-operation and death) in patients with Stage IA chondrosarcoma.
- 1.2 To assess local control and development of metastatic disease in patients with Stage IA chondrosarcoma when treated with intralesional resection.
- 1.3 To explore correlation of telomerase activity with chondrosarcoma grade in a preliminary fashion.
- 1.4 To explore if enchondromas can be differentiated from Grade 1 chondrosarcomas via hierarchical cluster (HC) analyses of cDNA microarray profiles in a preliminary fashion.

2.0 BACKGROUND

Stage IA chondrosarcoma (CS) of bone is defined as a low grade cartilage malignancy (Dorfman, Bone Tumors, 1998; Crim & Seeger, Radiology 1993; Sanerkin & Gallagher, JBJS-B: 1979; Schiller, SeminDiagn Pathol 1985) contained within bone (AJCC and Enneking staging system). Intralesional (IL) resection with margin expansion and local adjuvant for Stage IA CS has excellent local and systemic control rates. (2, 17) At minimum follow-up of 15 months, two and five years, the local recurrence in these series was a combined 3%, 3% and 4% respectively. Nevertheless, many centers still employ wide resection, a highly morbid procedure, for this disease. Some authors conclude intralesional surgery leads to high local recurrence. (15) In this latter study however, intralesional techniques were not described. While they had 3 of 3 patients with local recurrences at 24, 42 and 70 months, at the time of writing all were alive with no evidence of disease. Lee et al, report a subset of 86 patients with Grade 1 CS (but not necessarily Stage IA) with a local recurrence rate of 15% and commented that the margin of resection was not significant. (9) In all of these series, none of the Stage IA chondrosarcoma treated with intralesional resection developed metastases or died from disease. This controversy was highlighted in a review article in the Journal of the American Academy of Orthopaedic Surgeons. (Marco RA, et al. J Am Acad Orthop Surg 2000). At the Musculoskeletal Tumor Society Specialty Day during the 1999 American Academy of Orthopaedic Surgeons, treatment recommendation were equally bipartisan (wide versus intralesional) for Stage IA chondrosarcoma of bone. Because wide resection can involve significant local morbidity and may often be unnecessary it is important to answer the question as to whether Stage IA chondrosarcoma of bone can be managed exclusively with intralesional resection plus local adjuvant.

Inclusion of Women and Minorities:

This study was designed to include women and minorities, but was not designed to measure differences of intervention effects.

3.0 DRUG INFORMATION

There is not drug information for this study.

4.0 **STAGING CRITERIA**

DEFINITION OF TNM

Primary Tumor (T)

- T0 No evidence of primary tumor
- T1 Tumor 8 cm or less in greatest dimension
- T2 Tumor more than 8 cm in greatest dimension

Regional Lymph Nodes (N)

- N0 No regional lymph node metastasis
- N1 Regional lymph node metastasis

Distant Metastasis (M)

- M0 No distant metastasis
- M1 Distant metastasis
- M1b Other distant sites

Biopsy of metastasis site performed Yes No

Source of pathologic metastatic specimen _____

Histologic Grade (G)

- G1 Well differentiated - Low Grade
- G2 Moderately differentiated - Low Grade
- G3 Poorly differentiated - High Grade
- G4 Undifferentiated - High Grade ²

STAGE GROUPING

Stage IA	T1	N0	M0	G1, 2	Low grade
IIB	T2	N0	M0	G1, 2	Low grade
IIA	T1	N0	M0	G3, 4	High grade
IIB	T2	N0	M0	G3, 4	High grade
III	T3	N0	M0	Any G	
IVA	Any T	N1	M1a	Any G	
IVB	Any T	N1	Any M	Any G	
	Any T	Any N	M1b	Any G	

²Ewing's sarcoma is classified as G4

5.0 ELIGIBILITY CRITERIA

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. Use the spaces provided to confirm a patient's eligibility. For each patient, this section must be photocopied, completed and submitted to the Data Operations Center in Seattle (see Section 14.0).

SWOG Patient No. _____

Patient's Initials (L, F, M) _____

5.1 Patients must have suspected Stage IA chondrosarcoma as determined by the following (check all that apply):

_____ resorption of previous calcifications (change in radiographic appearance through time).

OR

At least 2 (but not more than 4) of the following:

_____ permeative appearance of medullary bone defined as presence of tumor around three sides of a trabeculae of normal bone

_____ endosteal scalloping (> 50% of cortex)

_____ cortical thickening

_____ bone "expansion"

_____ positive technetium scan

_____ 5.2 Patients must not have cortical disruption and/or soft tissue mass detected on imaging.

_____ 5.3 Patients must have radiographs, CT (3 mm cuts), and technetium bone scans completed within 42 days prior to registration.

Date disease assessed for:

Radiographs _____

CT _____

Technetium scans _____

Note: Recommended imaging protocol for CT scans include no contrast administration, bone and soft tissue algorithms, and coverage to include entire tumor as well as at least 4 cm on either side.

_____ 5.4 Patients must have a CT scan of the chest and chest X-ray within 42 days prior to registration:

Date of CT scan: _____

Date of Chest X-ray: _____

_____ 5.5 Patients must consent to have surgical specimens (see Section 12.0) submitted for central review.

SWOG Patient No. _____

Patient's Initials (L, F, M) _____

- _____ 5.6 Radiographs must be sent to central radiologist (see Section 15.0) for review by evaluating surgeon.
- _____ 5.7 Patients must be 18 years of age or older.
- _____ 5.8 Patients must not have had prior chemotherapy, radiotherapy, biologic therapy, or investigational anticancer agents for any reason for this tumor.
- _____ 5.9 Patients may not have received prior surgery (except biopsy). Biopsy will not be required.
Date of biopsy: _____ (N/A if not applicable)
- _____ 5.10 No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, adequately treated Stage I or II cancer from which the patient is currently in complete remission, or any other cancer from which the patient has been disease-free for 5 years.
- _____ 5.11 All patients must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines.
- _____ 5.12 At the time of patient registration, the treating institution's name and ID number must be provided to the Data Operations Center in Seattle in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered into the data base.

6.0 STRATIFICATION FACTORS

There are no stratification factors for this study.

7.0 TREATMENT PLAN

For study related questions, please contact Dr. Randall at 801/585-0300 or Dr. Biermann at 734/936-9594.

7.1 Good Medical Practice

The following pre-study tests should be obtained within 28 days prior to registration in accordance with good medical practice. Results of these tests do not determine eligibility and minor deviations would be acceptable if they do not impact on patient safety in the clinical judgment of the treating physician. The Study Coordinator must be contacted if there are significant deviations in the values of these tests.

Suggested pre-study tests and guidelines, if applicable, for good medical practice are as follows:

- a. CBC
- b. Patients should not be planning to receive other investigational agents, other chemotherapeutic agents, radiation therapy, or hormonal therapy while receiving treatment on this study except for steroids administered for antiemesis, adrenal failure, or septic shock or hormones administered for non-disease-related conditions (e.g., insulin for diabetes).

- 7.2 All potential Stage IA chondrosarcomas will be treated with intralesional resection (curettage with high speed burr) & local adjuvant (liquid nitrogen, phenol, alcohol). Minimal intralesional treatment is defined as follows:

NOTE: Surgery must be completed within 60 days of registration.

- a. Direct open surgical approach to site.
- b. Cortical fenestration of site, creating a window for the entire length of the lesion as determined by intraoperative fluoroscopy and/or preoperative radiographs, CTs. The width of the window should approximate at least 75% of width of the bone involved.
- c. Manual curettage.
- d. Margin expansion with high speed burr
- e. Documentation of margin expansion by technique preferred by surgeon (e.g. introduction of radiocontrast material or tool into created cavity under dynamic fluoroscopy). Final fluoroscopic images documenting extent of cephalad and caudad resection must be saved and submitted for central radiologic review.
- f. Administration of local adjuvant which may include liquid nitrogen, phenol or alcohol.
- g. Filling of the cavity may be either with polymethylmethacrylate cement or bone graft (allo of homograft acceptable).

- h. Lesions may be prophylactically fixed with plate and screws as needed. No intramedullary fixation permitted.
- i. Wound is closed over a Jackson-Pratt or similar drain.

Points a - i must be included and documented in the **S0344** Surgical Form (Form #).

7.3 **Criteria For Removal From Protocol Treatment:**

- a. Progression of disease (see Section 10.0).
- b. > 60 days from registration to surgery.
- c. The patient may withdraw from the study at any time for any reason.

7.4 All reasons for discontinuation of treatment must be documented in the Off Treatment Notice (Form #).

7.5 All patients will be followed for 5 years or until death (whichever occurs first).

8.0 TOXICITIES TO BE MONITORED

- 8.1 This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 3.0 for toxicity and Serious Adverse Event reporting. A copy of the CTCAE Version 3.0 can be downloaded from the CTEP home page (<http://ctep.cancer.gov>). **All appropriate treatment areas should have access to a copy of the CTCAE Version 3.0.**
- 8.2 Patients will be evaluated every three months for the first year and then once every six months for years 2 - 5. (see Section 9.0).

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 **Progression:** One or more of the following must occur: Unequivocal progression of disease in the opinion of the treating physician (an explanation must be provided). Appearance of any new lesion/site. Death due to disease without documented progression and without symptomatic deterioration (see Section 10.2).

10.2 **Symptomatic deterioration:** Global deterioration of health status requiring discontinuation of treatment without objective evidence of progression.

Notes: (1) In cases for which initial flare reaction is possible (hypercalcemia, increased bone pain, erythema of skin lesions), either symptoms must persist beyond 4 weeks or there must be additional evidence of progression. (2) Lesions that appear to increase in size due to presence of necrotic tissue will not be considered to have progressed. (3) For bone disease documented on bone scan only, increased uptake does not constitute unequivocal progression. (4) Appearance or worsening of pleural effusions does not constitute unequivocal progression unless cytologically proven of neoplastic origin.

10.3 **Local recurrence:** recurrence of enrollment radiographic criteria with subsequent histologic confirmation.

10.4 **Performance Status:** Patients will be graded according to the Zubrod performance status scale.

<u>POINT</u>	<u>DESCRIPTION</u>
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

10.5 **Progress Free Survival:** From time of registration to time of first documentation of progression (as defined in Section 10.1) or symptomatic deterioration (as defined in Section 10.2).

10.6 **Time to Death:** From date of registration to date of death due to any cause.

11.0 STATISTICAL CONSIDERATIONS

11.1 With 60 eligible patients, local recurrence probabilities toxicities can be estimated to within $\pm 13\%$. Any toxicity occurring with at least a 5% probability is likely to be seen at least once with 95% chance.

11.2 There is no formal data and safety monitoring committee for Phase II studies. Toxicity and accrual monitoring are done routinely by the Study Coordinator, study Statistician and the Disease Committee Chair. Response monitoring is done by the study Statistician and Study Coordinator. Accrual reports are generated weekly, and formal toxicity reports are generated every 6 months. In addition, the Statistical Center, Adverse Event Coordinator at the Operations Office, and Executive Officer monitor toxicities on an ongoing basis.

12.0 DISCIPLINE REVIEW

Central pathology review will occur *postoperatively* to confirm diagnosis. Eligibility will not be affected by review results. Blocks and slides must be submitted. Tumor specimens will also need to go to University of Iowa and Huntsman Cancer Institute for biologic studies (see Appendix 19.__).

Central pathology will evaluate:

- Nuclear features (pleomorphism, hyperchromatism)
- Mitotic rate
- Double nucleation
- Cytoplasmic features
- Cellularity (cells/hpf)
- Architectural pattern
- Frequency of abnormal, multinucleated giant cells

Central radiology review:

Initial plain radiographs, CT scans, technitium scans.

Intraoperative fluoroscopy images and immediatepostoperative radiographs.

Operative report to be submitted within 4 weeks of operation.

13.0 REGISTRATION

13.1 Patients must be registered prior to initiation of treatment (no more than 60 days prior to planned start of surgery).

13.2 For either phone or web registration, the individual registering the patient must have completed the appropriate Southwest Oncology Group Registration Form. The completed form must be referred to during the registration but should not be submitted as part of the patient data.

The individual registering the patient must also be prepared to provide the treating institution's name and ID number in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered into the data base. Patients will not be registered if the IRB approval date has not been provided or is > 365 days prior to the date of registration.

13.3 Registration procedures

- a. You may register patients from Member, CCOP and approved Affiliate institutions to a therapeutics study using the SWOG Registration program. To access the Registration program go to the SWOG Web site (<http://swog.org>) and click on the *Logon* link to go to the SWOG Members Area logon page (<https://swog.org/visitors/logon.asp>). This Web program is available at any time except for periods listed **under Down Times**. Log on as an Individual User using your SWOG Roster IDNumber and individual web user password. Help for the logon process may be found at <https://swog.org/visitors/logonhelp.asp>. After

you have logged on, click on the *Clinical Trials* link and then the *Patient Reg* link to go to the Entry Page for the Patient Registration program. If you are a Registrar at an institution with Internet access you are encouraged to register this way. For new users, the link to a "Starter Kit" of help files may be found by clicking on **Starter Kit link at the logon page.**

To register a patient the following must be done (in order):

1. You are entered into the Southwest Oncology Group Roster and issued a SWOG Roster ID Number,
2. You are associated as an investigator or CRA/RN to the institution where a registration is occurring, and
3. You are granted permission to use the Patient Registration program at that institution.

For assistance with points 1 and 2 call the SWOG Operations Office at 210/677-8808. For point 3 you must contact your Web User Administrator. Each SWOG institution has one or more Web User Administrators who may set up Web Users at their institution and assign permissions and passwords to these users. For other password problems or problems with the Patient Registration program, please e-mail webreghelp@crab.org. Include your name, Roster ID Number, and telephone number, when the problem occurred, and exactly what you were doing.

- b. If the Web Reg program is not used, the registration must be done by phone.

Member, Affiliate and CCOP Institutions

Registration by phone of patients from member, affiliate and CCOP institutions must be done through the Southwest Oncology Group Data Operations Center in Seattle by telephoning 206/652-2267, 6:30 a.m. to 4:00 p.m. Pacific Time, Monday through Friday, excluding holidays.

- 13.4 For either method of registration, exceptions to Southwest Oncology Group registration policies will not be permitted.
 - a. Patients must meet all eligibility requirements.
 - b. Institutions must be identified as approved for registration.
 - c. Registrations may not be cancelled.
 - d. Late registrations (after initiation of treatment) will not be accepted.

14.0 DATA SUBMISSION SCHEDULE

- 14.1 Data must be submitted according to the protocol requirements for **ALL** patients registered, whether or not assigned treatment is administered, including patients deemed to be ineligible. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.
- 14.2 Master forms are included in Section 18.0 and (with the exception of the sample consent form and the Registration Form) must be photocopied for data submission to the Data Operations Center in Seattle. Alternatively, data from approved SWOG institutions may be submitted on-line via the Web; see Section 14.3a for details.

14.3 Data Submission Procedures. Please select **one** option for submitting specific data. Data submitted electronically or via facsimile does **not** need to be followed up with a mailed version.

- a. Southwest Oncology Group Member Institutions, CCOPs, UCOPs and approved Affiliate institutions may submit data electronically via the Web by using the SWOG CRA Workbench. To access the CRA Workbench, go to the SWOG Web site (<http://swog.org>) and logon to the Members Area. After you have logged on, click on the *CRA Workbench* link to access the home page for CRA Workbench website. Next, click on the *Data Submission* link and follow the instructions. If you are a CRA at an institution with Internet access, you are encouraged to submit data this way. For new users, the link to a "Starter Kit" of help files may be found by clicking on the **Starter Kit** link at the Members' logon page.

To submit data via the web the following must be done (in order):

1. You are entered into the Southwest Oncology Group Roster and issued a SWOG Roster ID Number,
2. You are associated as an investigator or CRA/RN at the institution where the patient is being treated or followed, and
3. Your Web User Administrator has added you as a web user and has given you the appropriate system permissions to submit data for that institution.

For assistance with points 1 and 2 call the Operations Office at 210/677-8808. For point 3, contact your local Web User Administrator (refer to the "Who is my Web User Administrator?" function on the swog.org Members logon page). For other difficulties with the CRA Workbench, please email technicalquestion@crab.org.

- b. Alternatives to the CRA Workbench/web based data submission option are: submission via facsimile, surface, or express mail.

For facsimile submission: Member, CCOP, UCOP and approved Affiliate institutions may submit data via facsimile to 800/892-4007 or 206/342-1680 locally. Faxed data no longer requires the Data Submission Facsimile Cover Sheet. It is preferred that data be faxed with no cover sheet at all. Facsimile submission is the 2nd preferred option for data submission.

For surface or express mail: Group Member Institutions, CCOPs, UCOPs and approved Affiliate institutions must submit one copy of all data forms directly to the Southwest Oncology Group Data Operations Center in Seattle at the address below. Affiliates must submit (number of copies to be determined by the Group member) copies of all forms to their Group member institution for forwarding to the Southwest Oncology Group Data Operations Center in Seattle at the following address:

Southwest Oncology Group Data Operations Center
Cancer Research And Biostatistics
1730 Minor Ave, STE 1900
Seattle, WA 98101-1468

14.4 WITHIN 4 WEEKS OF REGISTRATION:

Submit a copy of the following:

- a. Section 5.0
- b. Baseline Tumor Assessment Form (Form #8988)
- c. Entry radiographs

14.5 WITHIN 4 WEEKS OF SURGERY:

Submit tumor specimen per Section 15.0 with a copy of the Specimen Submission Form (Form #1951) to Dr. Randall's office. A copy of the Specimen Submission Form (Form #1951) must also be submitted to the Southwest Oncology Group Statistical Center.

14.6 ONCE OFF ALL PROTOCOL TREATMENT SUBMIT EVERY 6 MONTHS FOR 2 YEARS AND THEN ANNUALLY THEREAFTER UNTIL 3 YEARS AFTER REGISTRATION:

Submit a copy of the Follow-Up Form (Form #61519) documenting required parameters as specified on the Study Calendar. Designated radiographs must also be submitted.

14.10 WITHIN 4 WEEKS OF PROGRESSION/RELAPSE:

If the patient progresses while on treatment, submit a copy of the Follow-Up Tumor Assessment Form (Form #9755) documenting date, site and methods for determining progression/relapse. Otherwise submit the Southwest Oncology Group Follow-Up Form (Form #61519).

14.11 WITHIN 4 WEEKS OF KNOWLEDGE OF DEATH:

If the death occurs while patient is on protocol treatment, submit the Cycle Specific Toxicity and Dosage Form (Form #), the Follow-Up Tumor Assessment Form (Form #9755), and the Notice of Death Form (Form #61554). If death occurs after off treatment, submit the Southwest Oncology Group Follow-Up Form (Form #61519), and the Notice of Death Form (Form #61554).

15.0 SPECIAL INSTRUCTIONS

Biologic Studies

Two biologic correlate studies will be performed. For Study #1, paraffin blocks will be sent to the University of Iowa from the institution at which the patient is being enrolled. Cartilage tumor that is either snap frozen or placed in RNA^{later}™ will be sent to the Huntsman Cancer Institute at the University of Utah for cDNA microarray hierarchical cluster analyses.

15.1 Study 1

Hypothesis: Telomerase immunohistochemical expression is correlates malignancy: Stage IA chondrosarcoma has significantly increased expression as compared to enchondroma.

Immunohistochemical Detection of Telomerase in Cartilage Neoplasms

In vitro transformation studies indicated that telomerase expression is activated during malignant transformation. An immunohistochemical analysis of archived pathology

specimens will be performed to determine if this change is associated with tumor recurrence or metastasis.

Approach:

Five micron-thick sections will be cut from paraffin blocks, mounted on Fisher SuperFrost Plus slides (Fisher Scientific Co), and dried overnight in a 56°C oven. The sections will then de-paraffinized in xylene and hydrated through a graded series of ethanol to distilled water. The endogenous peroxidase is quenched in 0.3% hydrogen peroxide for 15 minutes. The sections are rinsed in distilled water before antigen retrieval with 0.01% citric acid, pH 6.0, using a pressure cooker. The slides are then rinsed in tap water and 0.1% phosphate buffered saline, pH 7.4 (PBS). A blocking solution of 10% normal horse serum- 1% bovine serum albumin- 0.1% Tween 20 in PBS is applied for 40 minutes. Sections are incubated overnight in a humid chamber at 4°C in a 1:8 dilution of monoclonal antibody NCL-hTERT (Novocastra Laboratories Ltd) in blocking solution. Excess antibody is removed with three rinses of PBS before the addition of blocking solution for 40 minutes. Horse anti-mouse IgG (H&L) rat adsorbed (# BA-2001, Vector Laboratories Inc., Burlingame CA) diluted 1:250 in blocking solution is applied for 30 minutes. The sections are again rinsed three times in PBS before incubation in Vectastain ABC (Vector Laboratories, Inc., Burlingame CA) solution made according to manufacturer's directions for 30 minutes. The excess solution is removed with three rinses in PBS before the reaction product is visualized using the Vector DAB Peroxidase Substrate Kit (Vector Labs) following package directions. Sections are rinsed in water for 5 minutes, counterstained with a 0.02% light green solution, and dehydrated in graded ethanol, cleared in xylene, and mounted with a synthetic mounting medium.

Preliminary Results

Immunohistochemistry on cell cultures using the monoclonal antibody NCL-hTERT (Novocastra Laboratories Ltd) detected telomerase in the nuclei of known telomerase-positive cultures but not telomerase-negative cultures. Furthermore, the antibody distinguished telomerase-expressing cells from non-expressing cells in mixed cultures. The antibody was applied to normal, paraffin-embedded tissues, including skin, articular cartilage, lung, and muscle. As expected, telomerase-staining was restricted to basal cells of the skin, which were strongly labeled. No staining was seen in more superficial skin cells or anywhere in the other normal tissues.

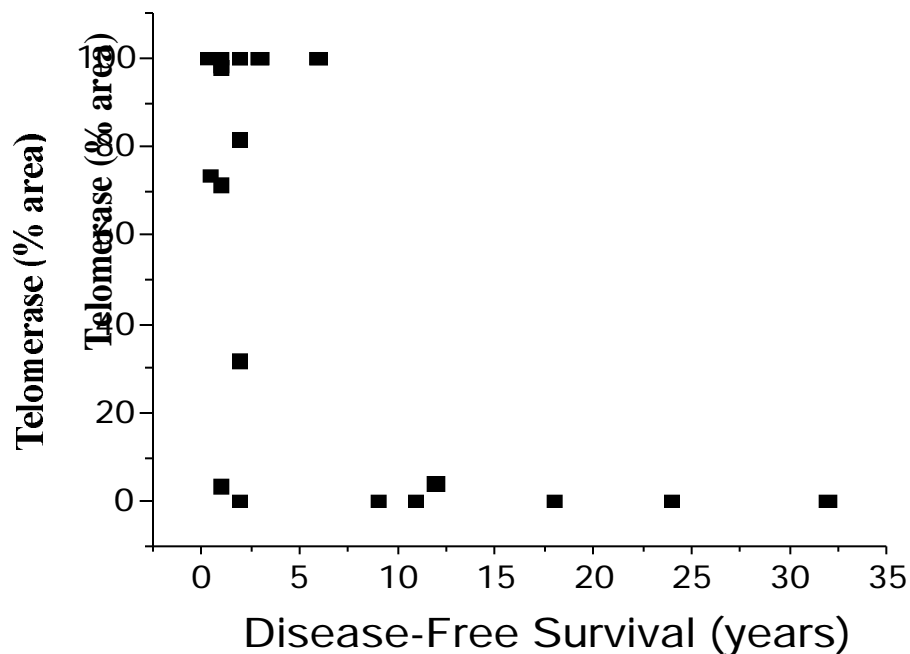
The anti-telomerase antibody was used to stain chondrosarcomas (Grades 1 - 3) and enchondromas. The intensity and nuclear localization of the stain, where it appeared, was similar in different specimens. Positive cells tended to be packed together in discrete colony-like patches, rather than interspersed with negative cells. Large positive areas dominated some specimens but, in others, positive cell areas were small (< 50 cells) and widely scattered.

We attempted to quantify the stain by counting positive cells in randomly-selected fields but found that even 20 fields/section under-sampled the sections to the extent that potentially significant numbers of telomerase-expressing cells were missed. Furthermore, the inherent "patchiness" of the stain distribution often led to a non-normal distribution for the positive cell counts, which tended to vary between 0% and 100% in different fields. To overcome these shortcomings we developed an alternative image analysis procedure. Our approach was feasible because positive cells were distributed in patches large enough to see at low magnification. Thus, it was practical to visually scan the whole section and accurately map the stained patches on a digitized, enlarged image. The entire area of the section and the portion occupied by stained cells was measured on a digitizing pad and these data were used to calculate % Positive Area. Normal tissue and acellular areas of tumors were excluded from the analysis so that the data reflect only the frequency of telomerase-staining cells in the tumor itself.

Telomerase staining results for a set of 59 cartilage neoplasms including chondrosarcomas and enchondromas are summarized in Figure 1 below and in Appendix 19. (Immunohistochemical Detection of Telomerase in Cartilage Neoplasms). Briefly, we found some telomerase expressing cells in most chondrosarcomas, but these varied in coverage from 1 - 100% in different tumors. Telomerase expressing cells were present to some degree in 77% of these specimens. Expression was significantly greater in chondrosarcomas than enchondromas ($p = 0.01$) and there was a trend toward increasing expression with increasing Grade ($3 > 2 > 1$) but the significance of this is unclear due to the relatively small numbers of Grade 2 and Grade 3 tumors. Most enchondromas were completely negative for telomerase but there were 2 exceptional cases that scored 100% positive. Although this indicated that telomerase was expressed at high-levels in benign cartilage tumors, the possibility that one or both of these were actually chondrosarcomas must be considered. Unfortunately, both of these "exceptional" cases were lost to follow-up and the original diagnoses are unverifiable.

We examined patient histories associated with the pathology specimens we had stained to determine if there was any correlation between telomerase expression and tumor aggressiveness measured in terms of the time intervals between recurrences (both local and metastatic). Of the 59 cases we examined, only 23 were documented sufficiently to calculate disease-free survival intervals. These included chondrosarcomas and enchondromas. In three cases no recurrences were described but follow-up times of 10 years or greater were available. Since these intervals were long enough for the patients to be considered cured of their tumors, we used their last follow-up dates as end points for disease-free survival. The % positive stain values for each tumor were paired with disease-free survival for that case. A plot of these data is shown (Figure 2). Statistical analysis (Pearson's product moment correlation) showed a significant negative correlation between these variables, supporting the hypothesis that the time to tumor recurrence decreases with increasing telomerase expression. In contrast, these times did not correlate with tumor grade ($p = 0.858$).

Immunohistochemistry is a robust method for analysis of telomerase expression in formalin-fixed, paraffin-embedded cartilage neoplasms. Moreover, the findings support the hypothesis that telomerase expression is related to malignancy. The stain may be particularly valuable in detecting potentially malignant tumors masquerading as benign tumors. In this regard we found that, among well-differentiated, Grade 1 chondrosarcomas, disease-free survival times in high telomerase expressers were significantly shorter than for low-expressers ($< 10\%$ positive) ($p = 0.001$). Despite this correlation we were unable to establish a specific "dose response" relation between telomerase expression levels and risk of recurrence due to the small size of the sample pool and paucity of tumors expressing intermediate-levels of telomerase. This underscores the need to analyze additional cases. Toward that end we will continue to recruit new cases in our own institution and work to expand our sample base by establishing collaborations with other institutions.



"Class discovery" and "class prediction" were first described by Golub et al, correlating gene expression profiles with tumor classification. Large-scale RNA assays will provide gene-expression data that will complement gene-sequencing data and greatly facilitate our understanding of sarcoma biology. Our investigations have validated cDNA microarrays as a technique for creating gene profiles of rare, heterogenous mesenchymal tumor samples ex vivo.

Tissue Handling/Specimen Preparation

Each participating institution will be provided with strict guidelines for tissue handling (Table) and a specimen handling package. This package will include one pre-measured (10cc) aliquot of RNA^{later}TM in a single 10cc sealed specimen container, two "wet" ice packs, and sealing tape in a 6 x 12 x 6 inch Styrofoam container. An overnight FedEx account (#1119604020) has been established and a preaddressed mailing slip will be provided in the specimen handling package. In our initial work, we established that mRNA is degraded if not placed in stabilizing solution within thirty minutes. Accordingly, specimens are to be handled by immediate placement in RNA^{later}TM and shipped overnight that day on ice packs in a Styrofoam container with a shipping invoice provided by us and overseen by our study coordinator. Each institution will have one package sent to them at a time. All specimens will undergo central pathologic review by Lester Layfield, MD.

A portion of the tumor is retrieved fresh and immediately placed in RNA^{later}TM (Ambion, Austin, TX) for 4°C, 48 hours maximum. As specimens for this study will be retrieved during curettage, one cm³ of tissue will be utilized for this study. Initial retrieval attempts revealed that in specimens that were not placed within 30 minutes into stabilizing solution, the quality of the mRNA was so degraded that insufficient material was obtained. Total RNA is isolated within 48 hours and quality is confirmed by agarose gel electrophoresis. We will use all the tissue available to isolate totRNA from the tissues. Tumors in excess of 1 gm are divided into aliquots up to 1g that are archived for later use at -70°C. From our preliminary data, 500 mg of tissue typically provides sufficient total RNA

to proceed with mRNA isolation or amplification. Tissue is minced and solubilized in Trizol for total RNA purification. The quality of the total RNA is confirmed with gel electrophoresis.

Although we have accumulated numerous mesenchymal tissues since the inception of our microarray project, our ability to run cDNA microarray analysis has been hindered by the fact that not all of our specimens have yielded sufficient mRNA to run arrays. We are currently using 2 μ g of mRNA to label with fluorescent dyes and hybridize to the microarray slides. We have set a cutoff of 230 μ g of total RNA from each specimen to proceed with mRNA isolation. 200 μ g is used for mRNA purification and 30 μ g is saved for possible amplification. Approximately 35% of our specimens accumulated to date adhere to this cutoff. While high grade tumors yield adequate RNA in excess of 50% of cases, low grade tumors are closer to 25%. Because cartilage tumors are relatively acellular, amplification will be necessary, as originally developed by Van Gelder et al. Briefly, this method employs synthesis of cDNA from total RNA from the sample or reference. The cDNA is used as a template to synthesize and amplify aRNA from the original sample. This method selectively amplifies mRNA since the cDNA is generated using an oligo dT primer. Theoretically, this amplification is linear, preserving the original expression level ratios of messages. (Arcturus Riboamp, Mountain View, CA). Therefore for this study, every specimen will be sufficient for microarray analysis as it will be amplified.

Microarrays and Analysis

Printing of slides: Our cDNA library was obtained from Research Genetics and is supplied as bacterial colonies in 96 well plates. These colonies are grown and purified. The inserts are PCR amplified using consensus sequence primers that recognize the plasmids used in the clone set. Agarose gel electrophoresis is run to check the PCR products that are subsequently purified by ethanol precipitation and resuspended in sodium citrate buffer. The clones are spotted on treated microscope slides using a robotic printer (Amersham Buckinghamshire, UK). Currently a single slide is formatted to contain 6,912 minimally non-redundant cDNA for which over 800 clones have been individually selected by Huntsman Cancer Institute investigators and represent genes primarily involved in transcription regulation, apoptosis, cell cycle control. The remaining clones are randomly selected from the clone set. Currently our studies have utilized a single slide to simplify our optimization and validation efforts. However, we plan to make use of all 4 slides generated by the microarray core facility bringing the total number of transcripts screened to 27,648. This should greatly enhance our ability to discover meaningful genes that correlate to diagnosis and prognosis.

Sample Hybridization:

cDNA hybridization probes are generated by reverse transcription of poly-A mRNAs and end-labeling with dCTP conjugated to fluorescent dyes. The hybridization probes will consist of the reference (Universal RNA) labeled with Cy-5 and the tumor samples labeled with Cy-3. The hybridization probes are diluted in buffer containing 1% (w/v) dextran sulfate, 50% (v/v) formamide, 0.3 M NaCl, 10 mM Tris pH 8.0, 1mM EDTA, 1x Denhardtts, 10mM DTT and 0,5 mg/ml non-homologous DNA. The probes are hybridized simultaneously with each other to the glass slides with the spotted cDNAs. After hybridization at 42°C for 36 hrs. in a humidified chamber, the non-bound probes are removed by washing with 2x SSC, 0.1% SDS at room temperature, followed by washing with 0.5x SSC, 0.1% SDS at 45°C. Fluorescence is read on the dried slides using a Molecular Dynamics Array Scanner (Amersham, Buckinghamshire, UK) at the appropriate emission wavelengths. Gene expression for each channel is quantified by ArrayVision software.

Data Normalization

Within each array and fluorescent channel (Cy-3 or Cy-5), the minimum value quantified is subtracted from each element to account for hybridization, dye incorporation, and background differences. Each gene is measured four times per hybridization (arrays are duplicated and two slides are used for each hybridization to provide four data points per cDNA) to improve reliability and eliminate false positives and negatives. The values from each of these measurements is averaged and then log₂ transformed to stabilize the variance and improve normality. These expression values represent how specific genes in the tumor are expressed relative to the reference and make up the profiles used to compare different tumors.

Sample Size

Although we cannot apriori determine the number of tumor samples we will need to determine statistically significant expression data to distinguish chondrosarcoma from enchondroma, in consultation with our biostatistical core, we feel the number of replicate tumor specimens per tumor type being evaluated should ideally be at least half the size of a target subset of genes that serve as a building block when determining a final set of differentially expressed genes. Accordingly, 10 or more specimens/tumor type may be necessary to truly validate our predictive power, however our preliminary results indicate that relative diagnostic categorization can be accomplished with as few as two to four specimens. This study will provide well in excess the number necessary.

Comparator

We will use the Universal Human Reference RNA available from Stratagene (La Jolla, CA) as our constant comparator for this study. Pooling the RNA from several different cells lines generates this universal RNA source. This results in mostly equal representation of most transcripts and a better global view of RNA expression in the tumor samples when hybridized competitively with this reference. It also allows better compatibility with other institutions that are also utilizing this as reference. The expense of this RNA source has prevented us from using this as a source for mRNA in the past but will be amplified and utilized in this study.

Data Analysis

One of the most daunting challenges facing scientists using microarray technology is dealing with the voluminous amounts of data generated by this technique. It is of paramount importance to be able to organize and visualize the data generated by microarray experiments in a way that provides meaningful and statistically significant interpretation. We currently use, as our primary tool, hierarchical clustering and principle component analysis, using classical supervised filtering methods (ANOVA) to select for discriminate genes that are differentially expressed in different tumor tissues. Using these supervised methods we have shown that we can successfully select a set of genes that can distinguish between different tumor groups. While supervised filtering methods have been useful, these methods have their limitations. Most importantly, supervised filtering requires grouping of the samples into related cohorts before performing the discriminate analysis.

We also use unsupervised approaches to look for discriminate gene patterns. For instance, we select genes that are expressed a minimum of 2 fold higher or lower compared to the reference samples in 10 - 25% of all samples. This enables us to generate discriminate gene lists without bias to pathological diagnosis.

Current use of hierarchical clustering and principle component analysis has allowed us to view the overall organization of the data so that major gene expression patterns and pathological groups can be identified. This preliminary approach has been useful in providing a framework for future studies. We will continue to implement these approaches and, in addition, we will utilize other visualization tools such as Self-Ordered-Maps and K-means clustering which use different mathematical algorithms to find discriminate gene patterns. All of these analytical tools are contained in the software program we have licensed from Spotfire Decision Site for Functional Genomics (Somerville, MA).

One of the most obvious deficiencies of the existing techniques is that they are essentially univariate and are frequently based on the (sometimes implicit) assumption that expression signals are stochastically independent. While univariate methods properly adjusted for multiple testing may be quite efficient whenever an abundance of differentially expressed individual genes is an inherent feature of the biological systems under comparison, there are many settings where gene-to-gene interactions are especially important so that multivariate methodology must be brought to the fore. Our previous studies have shown that by utilizing the correlation information multivariate methods, it is possible to find those genes whose differential expression is not detectable by univariate methods. In this project, we will use *SAM*, which is probably the best among known univariate techniques, and other univariate approaches to microarray data analysis, but invoking more general methods is clearly warranted. In view of considerable difficulties in formulating and testing an adequate multivariate statistical model for gene expression data, model-free statistical methods seem to be promising. As biostatistical analysis of microarray data is a process in evolution we will be collaborating intimately with the Division of Biostatistics at HCI to develop refined versions of these statistical procedures.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Publication and Industry Contact

The agent (hereinafter referred to as "Agent"), ZD1839, used in this protocol is provided to the NCI under a Clinical Trials Agreement (CTA) between OSI Pharmaceuticals (hereinafter referred to as "Collaborator") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines apply to the use of the Agent in this study:

1. The NCI encourages investigators to make data clinical trials fully available to Collaborators for review at the appropriate time (see #3). The NCI expects the clinical trial data developed under a CTA will be made available exclusively to Collaborator, and not to other parties.
2. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for cooperative group studies, or PI for other studies) of Collaborator's wish to contact them.
3. Any data provided to the Collaborator must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial should be provided to CTEP for immediate delivery to the Collaborator for advisory review and comment prior to submission for publication. Collaborator will have 30 days from the date of receipt for review. An additional 30 days may be requested in order to ensure that confidential and proprietary data, in addition to the Collaborator's intellectual property rights, are protected. Copies of abstracts should be provided to the Collaborator for courtesy review following submission, but prior to presentation at the meeting or publication in the proceedings. Copies of any manuscript and/or abstract should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
Executive Plaza North, Room 7111
Bethesda, Maryland 20892
FAX: 301/402-1584

The Regulatory Affairs Branch will then distribute them to the Collaborator.

Adverse Experiences

Any adverse experience, if deemed treatment related, must be reported to the Operations Office Adverse Drug Reaction (ADR) representative (210/677-8808), who will obtain information on the ADR. See guidelines below.

All adverse experiences must also be reported to the Institutional Review Board within 2 weeks and documentation of this report sent to the Operations Office.

All adverse experiences must also be recorded in the appropriate section of the case report form. The report should include, whenever possible, the investigator's written medical judgment as to relationship of the adverse experience to study medication(s) (i.e., "probable", "possible" or "unrelated").

Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31.

SWOG GUIDELINES FOR REPORTING OF SERIOUS ADVERSE EVENTS (SAE)

The following guidelines for reporting serious adverse events (SAE) apply to any Southwest Oncology Group protocol which utilizes a treatment modality other than chemotherapy. The following SAEs experienced by patients accrued to these protocols should be reported by telephone to the Operations Office (210-677-8808), within 24 hours of occurrence, to your Institutional Review Board (IRB) and by written notification to the Investigational Drug Branch, Cancer Therapy Evaluation Program, within 10 working days:

- (a) Any adverse event which is **both serious (life threatening, grade 4 or fatal, grade 5) and unexpected**.^{1,2,3} Occurrences of secondary AML or MDS must also be reported⁴.
- (b) Any increased incidence of an expected SAE which has been reported in the literature.
- (c) Any death on study if at least possibly related to the protocol treatment.

The SAE report should be documented on Form FDA-3500. Please mark "Not applicable" under Part C (suspect medications) and indicate the suspect treatment modality (i.e., radiation therapy, radiosurgery, etc.). Mail Form FDA-3500 to the address below:

Investigational Drug Branch
P. O. Box 30012
Bethesda, MD 20824

Send a copy of the Form FDA-3500 (or the NCI/CTEP Secondary AML/MDS Report Form if appropriate⁴), all data records and documentation of notification of your IRB to the Operations Office within 10 working days.

Southwest Oncology Group
ATTN: SAE Program
14980 Omicron Drive
San Antonio, TX 78245-3217

- See NCI Common Toxicity Criteria 2.0.
- Lists of expected adverse events can be found in the Introduction section, Section 8, and Informed Consent Form of the protocol.
- Adverse events judged unlikely or definitely not treatment related should not be reported. However, a report shall be submitted if there is a reasonable suspicion that the event is possibly, probably, or definitely treatment related.
- For reporting cases of secondary AML or MDS, please use the "NCI/CTEP Secondary AML/MDS Report Form" in lieu of Form FDA-3500.

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18.0 MASTER FORMS SET

This section includes copies of all data forms which must be completed for this study. These include:

- 18.1 Model Consent Form is included in this section. It must be reviewed and approved by the Institutional Review Board prior to patient registration.
- 18.2 Southwest Oncology Group Registration Form (Form #_____)
- 18.3 **S0344** Cycle Specific Toxicity Form (Form #_____)
- 18.4 Specimen Submission Form (Form #_____)
- 18.5 Southwest Oncology Group Baseline Tumor Assessment Form (Form #_____)
- 18.6 **S0344** Surgical Form (Form # _____)
- 18.7 Follow-Up Tumor Assessment (Form #_____)
- 18.8 Off Treatment Notice (Form #_____)
- 18.9 Southwest Oncology Group Follow-Up Form (Form #_____)
- 18.10 Notice of Death (Form #_____)

S0344, "A PHASE II SURGICAL TRIAL OF INTRALESIONAL RESECTION OF STAGE IA CHONDROSARCOMA OF BONE"

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your family and friends.

You are being asked to take part in this study because you possibly have CARTILAGE cancer.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to evaluate if less aggressive surgery will adequately prevent your cartilage tumor from returning to the spot from which it arose. Your tumor arises from within the bone and will be removed in a manner that is felt to be sufficient to entirely remove it but leaves the surrounding bone in place.

Alternatively, the entire bone could be removed, leaving a major defect requiring advanced reconstructive surgery. Furthermore, surgically removing the entire section of bone from which the tumor arose may be over treatment and result in significant risks and complications from the surgery itself. Preliminary data indicate that more conservative surgery, which you are considering in this study, will control the tumor just as well.

Your physician, as well as other orthopaedic tumor specialists, suspect that removing the entire bone is too aggressive and can put you at increased risk for functional disability.

After your tumor is removed via curettage or scraping out, it will be analyzed to see if it is cancerous. If it is not cancerous or a low grade or minimally aggressive cancer, no further treatment will occur. If it is a more aggressive tumor you will require more surgery as will be discussed by your treating surgeon. Your treating physician suspects that your cartilage tumor is not a highly aggressive type of cartilage cancer.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 60 people will take part in this study.

WHAT IS INVOLVED IN THE STUDY?

Your surgery will involve a less aggressive but still very thorough removal of the tumor. A window will be made in the bone and the tumor removed. To destroy any microscopic residual tumor cells an additional treatment will be employed. Your treating surgeon will discuss which technique is to be used and its benefits and risks. These include liquid nitrogen, ethanol, phenol or argon beam. Your surgeon

may wish to prevent the bone from breaking by applying a metal plate to strengthen the bone. It is important for you to know that the alternative treatment requires the removal of the entire segment of involved bone. To reconstruct the resulting bony defect has far greater risks of surgical complications than the technique being investigated in this study.

HOW LONG WILL I BE IN THE STUDY?

We think you will be in the study for up to 5 years or until the unlikely event that the tumor recurs.

The researcher may decide to take you off this study if your disease gets worse despite the treatment; the side effects of the treatment are too dangerous for you; new information about the treatment becomes available and this information suggests the treatment will be ineffective or unsafe for you.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for these side effects: tumor recurrence, infection, fracture, pain, injury to blood vessels and/or nerves. If you elect not to be in this study and undergo the more aggressive surgery all of these risks still apply and may even be increased. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict.

Risks and side effects related to the surgery we are studying include:

Pain-likely but treatable with pain medication

Infection- unlikely but possible

Fracture- unlikely but possible

Injury to blood vessels and/or nerves- unlikely but possible

Tumor recurrence- unlikely but possible

For more information about risks and side effects, ask the researcher or contact

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

We cannot and do not guarantee you will benefit if you take part in this study. The treatment you receive may even be harmful but your doctors feel this procedure is far less likely to cause surgical complications compared to more aggressive surgery. Your doctors feel that your participation in this study will give you at least as good a chance as you might expect from other treatments to cure you of your tumor. We

hope the information learned from this study will benefit other patients with cartilage cancer in the future.

WHAT OTHER OPTIONS ARE THERE?

Instead of being in this study, you have these options:

1. You may elect to forego surgical removal of your cartilage tumor. Your doctors cannot guarantee that at present the tumor seen in your studies is actually cartilage cancer although it is very suspicious. Because biopsies often misdiagnose cartilage cancers as benign (non-cancerous) a limited biopsy is not appropriate. Because potential cartilage cancer in cases such as yours is so slow growing you can elect to wait and watch with your physician to see how your tumor behaves. Radiographs (X-rays), computerized tomography (CT) scans, magnetic resonance imaging (MRI) and bone scans are all tools that your physician can use to look for disease progression. However, because you are being considered for this study your doctor feels that your tumor is very suspicious for cartilage cancer.
2. After talking with your surgeon, you may desire to have the entire segment of bone removed from which your tumor is arising. In certain cases this may actually be easier than the surgery that is proposed in this study but this is rare.

You can get treatment for cartilage cancer without being on this study. All of the treatment on this study may be available at this center or at other locations.

Please talk to your regular doctor about these and other options.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as: the National Cancer Institute, the Food and Drug Administration and the Southwest Oncology Group and Musculoskeletal Tumor Society.

If we publish the information we learn from this study in a medical journal, you will not be identified by name or in any other way.

WHAT ARE THE COSTS?

Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge.

(local institutions must choose one of the options below that best fits the hospital's situation:)

Funds have been set aside by the hospital to compensate you in the event of injury. Although no government or drug company funds have been set aside to compensate you for injury or illness, you do not give up any of your legal rights for compensation by signing this form.

-OR-

Although no funds have been set aside to compensate you for injury or illness, you do not give up any of your legal rights for compensation by signing this form.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

We will tell you about important new information that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the researcher NAME(S) at TELEPHONE NUMBER .

For questions about your rights as a research participant, contact the NAME OF CENTER Institutional Review Board (which is a group of people who review the research to protect your rights) at TELEPHONE NUMBER . *[And, if available, list patient representative (or other individual who is not on the research team or IRB).]*

WHERE CAN I GET MORE INFORMATION?

[To IRB/Investigators: Attach information materials and checklist of attachments. Signature page should be at the end of package. You may also wish to include the following informational resources]

You may call the NCI's Cancer Information Service at
1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web site...
www.cancer.gov

You will get a copy of this form. You may also request a copy of the protocol (full study plan).

SIGNATURE

You are deciding whether or not to take part in this study. If you sign, it means that you have decided to volunteer to take part in this study, and that you have read and understood all the information on this form.

Participant _____ Date _____

19.0 APPENDIX

19.1 Huntsman Cancer Institute Chondrosarcoma Microarray Project

19.2 Suggested protocol if MRI is obtained.

19.1 Huntsman Cancer Institute Chondrosarcoma Microarray Project

HUNTSMAN CANCER INSTITUTE CHONDROSARCOMA MICROARRAY PROJECT

Included in this package please find:

- A) Two ice packs
- B) One sealed 10cc specimen container containing 10 cc of clear fluid (RNAlater™ stabilizing solution)
- C) One Styrofoam shipping container
- D) One pre-addressed FedEx mailing slip

INSTRUCTIONS FOR TISSUE HANDLING OF SPECIMENS:

- 1) Upon receiving mailing packet, place ice packs in freezer.
- 2) At time of sterile tissue harvest, place specimen in provided RNAlater™ stabilizing solution provided in specimen container.
 - a) If obtained by an open technique, please provide 1 cm³ of tissue.
 - i. Slice the tissue into <5mm slices to maximize RNAlater™ diffusion
 - b) If obtained by core biopsy please provide 3 full cores (preferably 5).
- 3) Make sure the cap is TIGHT.
- 4) Immediately place specimen container in Styrofoam container with two frozen ice packs. Seal with enclosed tape and attach preaddressed Fed Ex mailing slip.

PLEASE NOTE: THIS MUST BE DONE < 30 MINUTES from time of tissue retrieval.

If RNAlater™ is not readily available, keep the specimen moist with STERILE saline and do NOT touch specimen with any nonsterile instruments. Enzyme contamination (e.g. on your washed hands) can degrade RNA quite readily.

- 5) **ENCLOSE A COPY OF THE IRB APPROVED SIGNED CONSENT FORM.**
- 6) Take to your institution's FedEx pick up ASAP in order to beat the pick up deadline.

If questions, concerns, or problems please contact Susie Crabtree, R.N. Study Coordinator, Sarcoma Service, at 801-581-5815 or pager 801-339-0339 24hours a day/7 days a week.

19.2 Suggested protocol if MRI is obtained.

IF MRI is obtained, suggested protocol:

Imaging of bone covering a distance extending at least 2 cm beyond in either direction;

Short axis through bone: Proton density, FSE T2 Weighted with fat saturation

Best long axis through bone: T1Weighted, FSEIR

Post-gadolinium: short and long axis T1-weighted with fat saturation

Time-activity curves following gadolinium injection useful if possible